

SHENTEK

EndoSynX™ Recombinant Cascade Kinetic-Chromogenic Assay (rCKA)

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE PERFORMING THE TEST

Product No.: 1501114

Version: A/0

For Research Use Only. Not for diagnostic or therapeutic use.

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■ Product Description

The **EndoSynX™ Recombinant Cascade Kinetic-Chromogenic Assay (rCKA)** is an advanced recombinant endotoxin detection system that mimics the enzymatic cascade of the natural *Limulus Polyphemus* Lysate using Recombinant Factor C, Recombinant Factor B, and Recombinant Proclotting Enzyme. Upon exposure to Gram-negative bacterial endotoxin (lipopolysaccharide, LPS), recombinant Factor C is activated, subsequently activate the serine cascade enzyme system that generates a kinetic chromogenic signal that correlates with the concentration of endotoxin. This fully recombinant protein cascade eliminated β -D-glucan induced Factor G interference in the traditional *Limulus* Amebocyte Lysate (LAL) assay, and made it suitable for the quantitative determination of endotoxin in pharmaceutical products.

The product described herein is for research use only and is not intended for diagnostic or therapeutic use.

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Storage
Recombinant Cascade Reagent A	PNR018	4 vials	2-8°C
Recombinant Cascade Reagent B	PNR019	4 vials	
Recombinant Cascade Buffer	PNR016	2 × 2.5 mL	
Control Standard of Endotoxin	PNB028	2 vials	
96-well Microplate	/	2 Plates	
Water for BET	NND072	4 × 8 mL	

■ Package Size

96 tests/Kit

■ Sensitivity

0.005 - 5.0 EU/mL

■ Shelf Life

12 months at 2-8°C. Please check the expiration date on the labels.

■ Additional Materials Required

- Sterile, pyrogen-free pipette tips: 10 µL, 100 µL, 200 µL and 1000 µL.
- Sterile, pyrogen-free glass tubes or penicillin bottles for serial dilutions of standard solutions or test samples.
- Sterile, pyrogen-free reservoirs: 50 mL

■ Additional Equipment Required

- An incubating kinetic microplate reader: 405 nm, kinetic reading and incubation at 37°C±1°C
- Calibrated automatic pipettes: 10 µL, 100 µL, 200 µL and 1000 µL
- Vortex-type mixer

Note: Laboratory materials that need to be endotoxin-free should be validated or certified to be less than the lowest endotoxin detection level of the test.

■ Maximum Valid Dilution (MVD)

The maximum valid dilution (MVD) is the maximum allowable dilution of a test sample at which the endotoxin limit can be determined.

$$\text{MVD} = (\text{endotoxin limit} \times \text{concentration of sample solution}) / (\lambda)$$

- λ = lowest concentration used in the standard curve, and it is 0.005 EU/mL of this kit.

For example, the endotoxin limit of antibody drugs is 0.1 EU/mg. If the lowest point of the standard curve is 0.005 EU/mL, where the concentration of Sample Solution is 10 mg/mL, the MVD equals 1:200. Thus, the antibody drugs can be diluted up to 1:200 to settle potential interference (inhibition or enhancement).

Endotoxin Limit

Endotoxin limit (EU/mL, EU/mg, EU/Unit) is calculated as: USP <85> and Ph. Eur. <2.6.14> have listed endotoxin limits of 5 EU/kg for intravenous drugs and 0.2 EU/kg for intrathecal drugs.

$$\text{Endotoxin Limit} = K / M$$

- K = threshold pyrogenic dose of endotoxin per kilogram of body mass
- M = maximum recommended bolus dose of product per kilogram of body mass
- For infusions: maximum total dose administered in a single-hour period.

Concentration of Sample Solution

- mg/mL if the endotoxin limit is specified by mass (EU/mg);
- Units/mL if the endotoxin limit is specified by unit of biological activity (EU/Unit);

- mL/mL if the endotoxin limit is specified by volume (EU/mL).

■ Kinetic-colorimetric Test Procedure

1. Set the microplate reader before use:

Table 2. Instrument settings (e.g. MD SpectraMax M2)

Metric	Parameter
Temperature	37°C
Reading type	ABS, dynamic detection
Shaking (before detection)	Medium speed, 5-10 seconds
Wavelength	405 nm
Reading time	60 minutes
Reading interval	30-60 seconds
Onset OD	0.02-0.1

2. Preparation of Standard Endotoxin Stock Solution

Reconstitute Control Standard of Endotoxin (CSE, PNB028) in Water for BET (NND072) according to the label instructions. Mix on a vortex mixer for at least 15 minutes to obtain a standard endotoxin stock solution with a final concentration of 100.0 EU/mL (recommended).

$$\text{Water for BET (NND072) (mL)} = \frac{\text{Control Standard of Endotoxin (CSE, PNB028) (EU)}}{100.0 \text{ EU/mL}}$$

For example, if the label indicates that a vial of Control Standard of Endotoxin (CSE, PNB028) contains 82.7 EU, adding 0.827 mL (827 µL) of Water for BET (NND072) yields a standard solution at 100.0 EU/mL.

3. Preparation of Standard Endotoxin Solutions

Use sterile, pyrogen-free glass tubes or penicillin bottles to dilute the standard endotoxin stock solution at the concentrations of 5.0, 0.5, 0.05, and 0.005 EU/mL. **Vortex vigorously for 2 minutes after each dilution.**

Table 3. Standard Endotoxin Solutions (Starting with 100.0 EU/mL as an example)

Final Conc. (EU/mL)	Water for BET (mL)	Previous Conc. (EU/mL)	Endotoxin Added (mL)
10.0	0.9	100.0	0.1
5.0	0.5	10.0	0.5
0.5	0.9	5.0	0.1
0.05	0.9	0.5	0.1
0.005	0.9	0.05	0.1

4. Test Samples Preparation

Prepare the test sample and positive product controls (PPC). The concentration of spiked endotoxin should follow the **Test for Interfering Factors** as below, equal to the concentration at or near the middle of the standard curve.

5. Loading Samples into Microplate

Add 100 μL of Standard Endotoxin Solutions, Water for BET (NND072, as negative control), test samples, and positive product controls (PPC) into the 96-well Microplate, respectively. Prepare at least 2 replicates for each sample.

Note: Each assay should include samples or dilutions of samples or products, positive product controls (PPC), either a series of dilutions covering the desired standard curve or a positive water control, and a negative control.

6. Preparation of Reaction Mixture Solution

- 1) Recombinant cascade reagent A solution (rCR-A): Dispense 0.75 mL of Water for BET (NND072) into the vial of Recombinant Cascade Reagent A (PNR018) and gently dissolve the powder until the solution clears.
- 2) Recombinant cascade reagent B solution (rCR-B): Dispense 0.75 mL of Water for BET (NND072) into the vial of Recombinant Cascade Reagent B (PNR019) and gently dissolve the powder until the solution clears.
- 3) Calculate the number of reaction wells to decide the volume of Reaction Solution (including the pipetting loss).

Table 4. Preparation of Reaction Mixture Solution

Wells	Recombinant Cascade Buffer (μL)	rCR-A (μL)	rCR-B (μL)	Total (μL)
1	50	25	25	100
24	1300	650	650	2600
48	2500	1250	1250	5000

Note:

- 1) Each vial of reagent needs to be equilibrated to room temperature before use.
- 2) Addition order: Recombinant Cascade Buffer (PNR016) \rightarrow Reagent A solution \rightarrow Reagent B solution.
- 3) Mix by gentle inversion (no vigorous shaking).

7. Add Reaction Mixture Solution

Add 100 μL of Reaction Mixture Solution to each well and insert the plate into microplate reader immediately.

Note: To ensure the accuracy of the test, the dispensing of Reaction Mixture Solution into each well of the 96-well Microplate must be completed within 5 minutes.

8. Start the test

Run using preset kinetic parameters of microplate reader.

■ Data analysis

1. Standard Curve

The relationship between start-up time (T) and log concentration (C)

$$\lg T = b \lg C + a$$

- b = slope
- a = intercept.

2. Calculation of Sample Concentration

Determine the endotoxin concentration by interpolation from the standard curve.

■ Test for Interfering Factors

Select an endotoxin concentration at or near the intermediate endotoxin standard curve. Prepare Solutions A, B, C and D as shown in Table 5. It is recommended that the interference test for Solutions A, B, C and D be performed on at least two replicates.

Table 5. Preparation of Solutions for the Inhibition / Enhancement Test

Solution	Endotoxin Concentration	Solution to Which Endotoxin Is Added	Number of Replicates
A ^a	None	Sample Solution	≥ 2
B ^b	Middle concentration of the standard curve	Sample Solution	≥ 2
C ^c	At least three concentrations (lowest concentration is designated as λ)	Water for BET	≥ 2 each
D ^d	None	Water for BET	≥ 2

^a Solution A: The Sample Solution may be diluted, but must not exceed the MVD.

^b Solution B (positive product control): The concentration at the same dilution as Solution A, containing a given concentration of endotoxin equal or near to the intermediate concentration of standard curve.

^c Solution C: Standard curve, we recommend to prepare Standard Endotoxin Solutions from 0.005-5.0 EU/mL.

^d Solution D: Water for BET (NND072, negative control).

The test is considered valid when the following conditions are met:

1. The absolute value of the correlation coefficient generated by the standard curve using Solution C is greater than or equal to 0.980.

2. The result with Solution D is less than the endotoxin detection limit of the recombinant reagent employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution, if any (Solution A), from that containing the added endotoxin (Solution B). In order to be considered free of factors that interfere with the assay under the conditions of the test, the measured concentration of the endotoxin added to the Sample Solution must be within 50%–200% of the known added endotoxin concentration after subtraction of any endotoxin detected in the solution without added endotoxin.

■ Warnings and Precautions

1. For Bacterial Endotoxins Tests only.
2. Use endotoxin-free materials and avoid contamination.
3. Discard the dissolved Recombinant Cascade Reagent A (PNR018) if the solution turns yellow or any insoluble substances appear.
4. The pH value of the Sample must be 6.0-8.0.

Caution: *As a novel assay, it is the responsibility of the user to review the supplier's primary validation package and to verify that the recombinant reagent-based. This verification must include performing specific experiments to method is appropriate for use in testing specific products or materials confirm that the method is suitable for its intended purpose under the conditions of use for the material, drug substance, and/or drug product. The user should refer to Verification of Compendial Procedures <1226> and the Verification-Test for interfering Factors section.*

■ References

- USP <85> Bacterial Endotoxins Test
- USP <86> Bacterial endotoxins test using recombinant reagents
- USP <1226> Verification of Compendial Procedures
- Ph. Eur. <2.6.14> Bacterial Endotoxins

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Support & Contact

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